

Spontaneous Cancer in Mice.

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The present paper is based upon observations and experiments made on 300 mice, obtained in the laboratories of the Imperial Cancer Research Fund since the summer of 1908, and in which cancer had developed spontaneously. Excluding lung adenomata, lymphomata, and sebaceous adenomata there remain 288 mice in which 350 tumours were observed, microscopical examination showing that they were to be regarded as true new growths. When these tumours were first observed, *i.e.* at the time of entering them on the laboratory register, 49 of the mice presented multiple tumours, and in an additional 77 mice, other subcutaneous tumour-nodules developed at a later date. Of the 350 tumours first observed, 42 had a structure quite distinctive from that of the well-known mammary tumours of the mouse, and of them 25 had certainly arisen outside the mamma; 22 were squamous-celled carcinomata with marked keratinisation, and of them 14 arose in the mammary region, and 8 entirely outside it (jaw, face, vulva, and anus); 5 were sebaceous carcinomata, and of them 3 arose in the mammary region, and 2 outside it (anus).

The remaining tumours were:—

- 2 Carcinomata of the preputial gland.
- 2 Adeno-carcinomata of the kidney (1 a hypernephroma?).
- 2 " " ovary.
- 6 Sarcomata (4 spindle cell, 1 round cell, 1 polymorphous cell).
- 1 Spontaneous "mixed" tumour.
- 1 Melanoma.
- 1 Fibro-myoma uteri.

(Not included in the above figures are 30 lung adenomata observed in 19 mice and cases of lymphomatous tumours.)

All the other tumours were mammary adeno-carcinomata.

The observation of typical malignant new growths in different organs provides new and additional evidence of a general liability of the tissues of the mouse to cancer, *i.e.* of an incidence in anatomical sites as varied as in man. Their malignant nature was evidenced by their clinical course, their recurrence after operation, the formation of metastases, and the results of

transplantation. In short, cancer in the mouse is essentially the same process as in man.

There are, however, certain characteristic features of its incidence in the mouse attracting attention and demanding an explanation. The enormous preponderance of tumours of special organs, in particular the frequency of growths of the mamma, constitutes one of them. The frequency of multi-centric development stands out as a further characteristic feature of tumours of the mamma in this animal. The data accumulated point towards an increased liability of the whole mammary system of the mouse, either as an inherited condition, or as the result of a general diseased condition, with diffuse changes in the mamma, leading to development of cancer. The same consideration applies to the adenomatous nodules in the lungs; they are also remarkably frequent and most often multiple, and to a less extent the same considerations apply to the lymphomatous tumours occurring in the mouse.

It has been generally assumed that the conditions of life of the mouse—in particular, factors connected with the physiological demands made upon the mamma, through the constantly repeated pregnancy and lactation—would suffice to account for the frequency of the mammary tumours. This point has been investigated experimentally, and the previous history of a number of mice spontaneously attacked by cancer, which have been observed since birth in the laboratory, yields no evidence of these physiological factors playing any part.

The records of the breeding experiments of the laboratory with regard to the previous history of the mice which have developed spontaneous cancer of the mamma give the following data:—Of 74 mice which had developed mammary tumours, and which were under observation from birth, only 33 are recorded to have littered previously to the development of the tumour, while no litter is recorded for 41. Analysing this latter figure, there is conclusive evidence that 20 mice have been completely isolated since quite young, and have never been with a male; and whilst the other 21 have not been so strictly isolated, but may at some time or other have been in a cage along with a male, none of them have littered. Of the 33 mice which had littered previously to the development of the tumour:—

1	litter is recorded in	11	cases.
2	litters are recorded in	7	„
3	„	7	„
4	„	5	„
5	„	2	„
7	„	1	case.

These figures show that it is hardly likely that excessive physiological demands made upon the mamma determine the development of tumours in this organ, but that other factors must be looked for.

The mammae of a large number of old mice, cancerous and non-cancerous, have been examined histologically in serial sections, in order to investigate whether changes might be found which could account for the frequency and the multicentric origin of the tumours.

Pathological changes are very frequently met with, both in the interstitial tissue and in the epithelium. The former occur either diffusely or strictly localised, and are more or less of a chronic inflammatory character; the epithelial changes are either hypertrophic or cystic in character. The interstitial and epithelial changes are often combined. A generalised or circumscribed hypertrophic condition of the mammary epithelium occurs frequently. A general hypertrophic condition mostly indicates physiological activity following on pregnancy, and has no interest for the present purpose. Now and then such hypertrophic changes are found in a mouse which is beyond the age when a physiological activity of the mamma is to be expected, and when the mouse has been kept isolated for five or six months without a male.

The hypertrophic condition, when circumscribed, has a special interest because of the similarity of the lesions to early tumour formations. All degrees of the process are found, from a slight increase in the number of the normal acini forming one lobe to definite hypertrophic nodules. On the other hand, all gradations exist between such hypertrophic nodules and true tumours, and it is often impossible to decide between the alternatives of a hypertrophic nodule and a commencing tumour. These changes in the gland epithelium are generally accompanied by a more cellular connective tissue. They are very often multiple, and then occur either in the same mamma or in several mammae from the same individual.

The frequency of interstitial changes—inflammation and sclerosis—in the mammae of old mice of the laboratory is striking. There is evidence that the presence of nematodes in the subcutaneous tissue may be made responsible—at least to a large extent—for them. In several cases the interstitial changes co-exist with typical nodular hypertrophy of the epithelium, and this association suggests that the different pathological changes, inflammatory processes, sclerosis and cellularity of the connective tissue, and the nodular hypertrophy of the epithelium may be linked together, and referred to a common cause, the presence of nematodes in the subcutaneous tissue.

Thus, pathological changes of an inflammatory character are very

frequent in the mammary gland of old mice. These changes have been entirely overlooked, up to the present, in the mammary gland of this animal. Along with the interstitial changes followed by sclerosis, hypertrophic changes of the glandular epithelium are frequent. In some cases the evidence favours the view that the epithelial changes are intimately connected with interstitial lesions, in others the connection cannot be definitely established. In a large number of instances the hypertrophic condition of the epithelium appears to be the basis on which tumours develop. The suggestion is made that the interstitial lesions may play an essential part as a mediate cause of the epithelial changes. An explanation of the interstitial changes described may be afforded by nematodes which have been found living in the interstitial tissue where their embryos are liberated. They are capable of causing considerable inflammatory reaction, and many of the histological pictures of inflammation and sclerosis, so often found in the mamma of old mice, can with great probability be referred to their presence. The hypothesis is put forward that the frequent and multicentric inflammation throughout the subcutaneous interstitial tissue in which the mamma is embedded may explain the frequency and the multicentricity of the epithelial hypertrophic changes and of the tumours arising on them. Similar considerations apply to the tumours of the lung and perhaps also to the lymphomatous conditions.

Numerous experiments have been made in order to define the relative parts played by local causes, such as those described above, or by constitutional anomalies of the animal in the development of cancer, or, in other words, to obtain more exact information on the relations obtaining between the tumour-cells and the animal in which they arose. The observations thus made may be considered under the following heads:—

- (a) Inoculations into the same mouse in which the tumour originated (autologous or autoplasmic implantation).
- (b) Inoculations into other spontaneously attacked mice.
- (c) Inoculations into normal mice, young and old.

(A) *Autologous Implantation.*

The tumours employed and the results obtained are:—

	Positive.	Negative.
51 adeno-carcinomata of mamma—		
46 subcutaneous	44	2
5 intraperitoneal	5	
2 adeno-carcinoids	2	
5 squamous cell carcinomata	5	
1 sebaceous carcinoma	1	
3 spindle-celled sarcomata...	3	
1 melanoma	1 ?	

Out of 59 epithelial tumours of varied histology, 57 have grown on transplantation of the cells into the same spontaneously affected animal.* Of non-epithelial tumours there were four, three spindle-celled sarcomata which gave positive results on re-inoculation, for the fourth (a melanotic tumour of peculiar structure and of very low power of growth) no increase was noted of the grafts re-inoculated simultaneously in four different places into the mouse in which it had arisen, although the tumour cells remained alive for four months.

(B) *Inoculations into other Spontaneously Affected Animals.*

Of the tumours which have been re-implanted into the same mice in which they have arisen, a number have also been tested in other mice spontaneously attacked with cancer. The method of implantation, dose, and site were exactly the same as for the autologous implantations.

Fifty-eight implantations have been made, and of them five have been successful. The tumours employed and the details of the results obtained, as compared with the results in normal mice, will be seen from table appended.

(C) *Inoculations into Normal Mice, Young and Old.*

In all, 73 tumours have been transplanted into 7706 mice, of which 5519 have survived four weeks; 307 tumours have been obtained, *i.e.* an average of about 5.6 per cent.

The experiments recorded above show that, whereas the spontaneously attacked animal offers a much more favourable soil for the growth of its own tumour cells, it is very doubtful whether it offers any better conditions for

* The success attending autoplasmic implantation is not limited to tumours of the mouse. It affords a method for obtaining fresh material wherever difficulties arise in transferring the tumours to normal animals, especially valuable in the case of larger animals or with ulcerated tumours, and is thus practised in the laboratory.

Nature of tumour transplanted, and protocol number of the mouse bearing it.	Into mouse itself.	Into other spontaneously affected mice.	Into Normals.	
			Young.	Old.
Canceroid..... 266	+	0/2	0/349	
"..... 273	+	0/1	0/337	
"..... 349	+	1/7	5/180	
"..... 357	+	0/4	1/59	
Sebaceous carcinoma..... 292	+	0/3	4/42	
Adeno-carcinoma. Mamma 282	+	2/4*	4/26	0/12
"..... 322	+	2/7	8/52	
"..... 334	+	0/5	0/16	0/16
"..... 338	+	0/5	2/60	0/15
"..... 351	+	0/1	0/35	
"..... 353	+	0/1	3/35	
5 other adeno-carcinomata 5	+	0/10	Not tested	in normals
Spindle cell sarcoma..... 460	Not transplanted ; mouse killed	0/8	16/130	0/15
Total.....	16/16	5/58	43/1321	0/58

* 2/20 when all inoculations are reckoned.

growth of other spontaneous tumours than do normal animals. The question of the suitability of the soil afforded by cancer-mice to the growth of tumours is a most important one. In order to study this question a little more closely, the behaviour of cancer-mice to different transplantable tumours has been investigated. Five experiments with 46 spontaneously affected mice have been undertaken with four different tumours representing different types of growth.

In order to employ control animals as comparable as possible to the cancer-mice, cancerous mice from the breeding experiments of the laboratory have been used, and as controls, their sisters and other near relatives kept under exactly the same conditions and of the same age as the cancerous mice.

The results of a typical experiment are seen from the chart (fig. 1), done with a rapidly growing carcinoma which usually grows progressively in a high percentage (Carcinoma 63). Expressed in figures, the result is, in young normals, six tumours in ten mice, *i.e.* 6/10; in old normals, 5/10; in old non-cancerous females of the breeding experiments of exactly the same ages as the cancerous mice, 2/10 (*plus* three positive cases which died too early to settle definitely whether the tumour would have grown progressively); and in cancer-mice from the same breeding experiments, 4/7.

On the whole, these experiments have failed to elicit any striking difference in suitability between spontaneously affected and normal animals. As in all transplantations of tumours, young animals show a greater susceptibility than older ones. In most of the experiments the transplanted

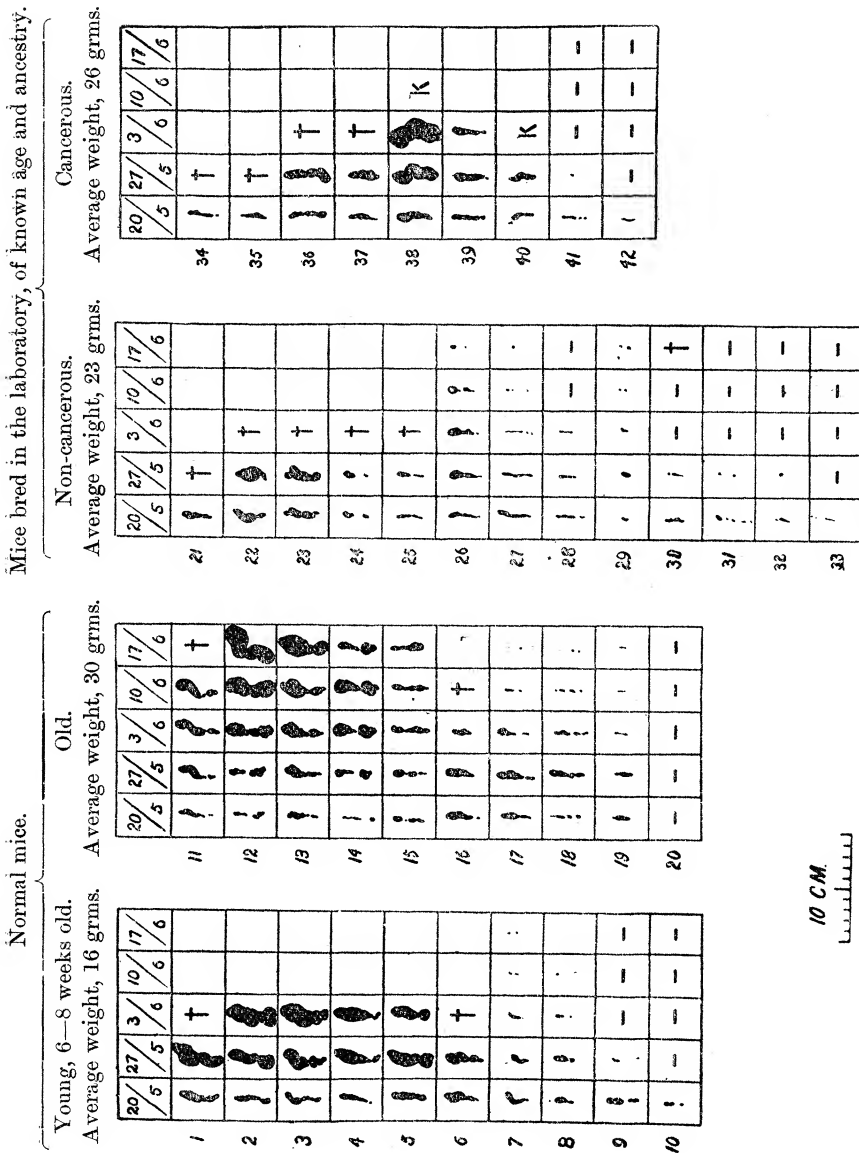


FIG. 1.—Suitability for the Growth of Transplanted Cancer of Mice in which Cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice. All mice inoculated 10.5.10 in the right axilla with 0.02 c.c. of Carcinoma 63. (Exp. 63/45 B.) Mice 1—33 controls (see headings of columns); 36—42 cancerous mice.

tumours have grown better in the young control mice than in the spontaneously affected animals. When the cancer-mice are compared with old mice of the same age, there seems to be a difference in some experiments in the case of certain tumours in favour of the former. This is the case in the experiment recorded on the chart (fig. 1). A similar difference seems to be present in the results of implantation of some spontaneous tumours (*vide* Table, p. 537). In other experiments, hardly any such difference can be discovered,

while in some the growth in the spontaneously affected mice was behind that in the old normal mice. The differences which exist in the two first-mentioned experiments are small, and, keeping in view the fact that the conditions in spontaneously affected and in normal mice, even at the best, are not strictly comparable, there does not seem to be evidence of an essentially enhanced suitability for growth of cancer cells in general, in mice spontaneously affected with cancer as compared with normal mice.

The experiments show that growth nearly always follows when a fragment of a spontaneous tumour is re-introduced into the animal in which the tumour has arisen, whereas the result is positive only in a very small percentage of normal animals. A general or constitutional idiosyncrasy favourable to the growth of its own cancer seems to be present in the spontaneously attacked animal, but it only applies to cells of its own body and not to cancer cells in general. On investigating spontaneously affected cancer-mice from the standpoint of the soil they offer for the growth of tumour cells which have originated in other individuals, one finds that the conditions then do not differ much from those obtaining in normal animals. The same inference can be drawn from the results obtained when cancer-mice are tested as to their suitability for the growth of propagated tumours, and the result compared with that obtained in normal mice under conditions as similar as possible. The comparison demonstrates that there is no evidence of general constitutional changes in the spontaneously attacked animals, greatly favourable to the growth of cancer cells in general, which can explain the appearance of a cancer. The results of autologous implantations are to be explained by the fact that the cells introduced belong to the same organism, where they have the full command of the supply of vessels and supporting tissues, and because they can be reintroduced either without setting up any inimical reaction, or if a reaction directed against them is set up, being in congenial surroundings, they are indifferent to it.

Experiments have been made to ascertain if reactions similar to those observed in normal mice against propagated tumours take place in spontaneously attacked mice, and if they do, whether they are efficient against their own tumour. Cancer-mice are capable of bringing transplanted tumours to absorption in the same way as normal mice do, and there is no reason to assume that the reactions leading to absorption differ essentially in spontaneously attacked mice and in normal mice, when the tumour cells introduced are derived from another individual. In some rare instances there is evidence that a process of spontaneous absorption or healing may also take place in a mouse towards its own spontaneous tumours, but it is very rare, and the evidence points rather to the

importance of local than to general or constitutional changes. When an attempt is made to induce increased resistance in spontaneously attacked mice, by the same means as are successful in normal mice against implantation of cancer, they seem to be totally ineffective against the implantation of the spontaneous tumour in the mouse in which it has arisen. There seems to be an insensibility of the cell in its own animal to reactions which are effective against strange cells in the same surroundings.

The outstanding result of this inquiry is the production of evidence of possible *local causes* of origin, on the one hand, and the absence of *general constitutional changes* favourable for growth on the other. The importance of local factors for other forms of cancer has long been recognised, and has been drawn attention to repeatedly in previous publications of the Imperial Cancer Research Fund. As knowledge of them increases, their many-sided nature appears more and more clearly. Whether they be animate or inanimate, bilharzia, nematodes, growth of other cancer cells as in the development of sarcoma as in Mouse 469, or mechanical, chemical, and actinic influences, the ultimate result of their action may be the same—the creation of new cell strains with powers of continuous growth. On the one hand, this process of cancerous transformation shows a close association with chronic irritative changes; on the other it has many points in common with what is known as spontaneous variation, giving rise to sports in animals or plants. The part played by the chronic irritant is obviously a mediate one, either so that it produces the altered conditions under which the first departure of the cell from the normal may take place, or by giving spontaneously occurring sports of cells opportunity of multiplying and by degrees adapting themselves to a new mode of life, similar to what is observed in propagated tumours. This hypothesis rests on the assumption that the malignant transformation of the tissues may take place by stages, for which there now seems to be ample evidence both from the facts obtained from observation of spontaneous cancers and from those obtained in propagated tumours. The nature of this process is still unknown, but it is possible that it is being elucidated indirectly by the study of the variations which, as recorded in another paper, have been demonstrated to occur in cancer cells during their continued propagation. The hope of being able to investigate the nature of the process by more direct methods lies in the possibility of being able to reproduce experimentally the conditions under which it may take place.
